UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Chemical Safety
and Pollution Prevention

DATE: June 5, 2013

**TXR# 0056658**

MEMORANDUM

SUBJECT: **Fenamiphos**: Review of Comparative Cholinesterase Assay (CCA) Draft Protocols

**PC Code:** 100601
**Decision No.:** 478458
**Petition No.:** none
**Risk Assessment Type:** none
**TXR No.:** 0056658
**MRID No.:** none

**DP Barcode:** 411617
**Registration No.:** none
**Regulatory Action:** none
**Case No.:** NA
**CAS No.:** 22224-92-6
**40 CFR:** NA

FROM: Ronnie J. Bever Jr., PhD, DABT
Registration Action Branch VII
Health Effects Division (7509P)

THRU: Anna Lowit, Ph.D.
Immediate Office
Health Effects Division (7509P)

Michael Metzger, Branch Chief
Registration Action Branches V and VII
Health Effects Division (7509P)

TO: Eric Miederhoff, Risk Manager Reviewer
Michael Goodis (RM 53), Risk Manager

**I. CONCLUSIONS:** Three draft protocols for the fenamiphos comparative cholinesterase assay (CCA) and five supporting studies have been reviewed. The protocols and studies were submitted by the AMVAC Chemical Corporation on May 9, 2013 and are detailed below. If AMVAC has questions, HED is willing to discuss them via a teleconference.

additional studies (listed below) were submitted concurrently. PRD requested review of three draft protocols for the fenamiphos comparative cholinesterase assay, considering the five supporting studies that were concurrently submitted. The submissions are listed below:

Donoghue, K. (2012) Fenamiphos Technical: Development and Validation of Analytical Methods and Liquid and Diet Formulation Preparation, Homogeneity and Stability. Project Number: BDG0161. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 46p. MRID 49067201.

Leggett, A. (2013) Fenamiphos: Single and Repeat Exposure Dose Range Finding Study in Male and Female Juvenile Crl:CD(SD) Rats Crl: CD(SD) Rats by Oral Gavage Administration. Project Number: BDG0163. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 93p. MRID 49067202.

Leggett, A. (2013) Fenamiphos: Single Dose Time to Peak Effect Oral Gavage Study in Young Adult Female Crl: CD(SD) Rats by Clinical Observations and Cholinesterase Analysis. Project Number: BDG0164. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 76p. MRID 49067203.

Leggett, A. (2013) Fenamiphos: Single Dose Time to Peak Effect Oral Gavage Study in 11 Day Old Juvenile Crl: CD(SD) Rats by Clinical Observations and Cholinesterase Analysis. Project Number: BDG0165. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 86p. MRID 49067204.

Leggett, A. (2013) Fenamiphos: Single and Repeat Exposure Dose Range Finding Study in Young Adult Female Crl: CD(SD) Rats by Oral Gavage Administration. Project Number: BDG0183. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 92p. MRID 49067205.

The 3 protocols submitted for consideration included: (i) repeat dose comparative sensitivity study in young adult and 11 day old juvenile Crl:CD (SD) rats by oral gavage administration (HLS study # BDG0168); (ii) single dose comparative sensitivity study in young adult and 11 day old juvenile Crl:CD (SD) rats by oral gavage administration (HLS study # BDG0167); and (iii) gestational exposure cholinesterase inhibition study in Crl:CD (SD) rat by oral administration (HLS study # BDG0166).

## III. EPA COMMENTS/DISCUSSION

### a. Time to Peak Effect

Time of Peak effect in PND 11 rats was shown to be 2-8 h in males and females for both RBC and brain acetylcholinesterase (CHE) inhibition (MRID 49067204). During this time interval, CHE inhibition was 82-86% in RBCs and 69-81% in brain. Animals (both sexes) were treated at 0 or 3.6 mg/kg (n=3 per time point of each dose). At this dose 4 hours after treatment, 6/9 treated males and 3/9 treated females displayed tremor, and no deaths occurred.

The submitted protocol suggests 8 hours for the time to peak effect; however, EPA considers 4 hours as an appropriate time to peak effect for juveniles. The 4 hour time point

was selected because response is at a plateau over the 2-8 hour period and lower doses sometimes result in earlier time of peak effect and a quicker resolution than the observed 6 hour plateau.

Time of Peak effect in adult female rats was shown to be 1 h for both RBC and brain CHE inhibition (MRID 49067203). At 1 h, CHE inhibition was 88% in RBCs and 17% in brain. Similar CHE inhibition results (80% in RBCs and 16% in brain) were noted at 2 h. Animals (females only) were treated at 0 or 5.6 mg/kg (n=3 per time point of each dose). At this dose, one treated animal died and 4/11 surviving treated animals had tremor at approximately 80 minutes after dosing. The submitted protocol suggests 1 hour for the time to peak effect, and the EPA concurs.

**b. Dose Selection Rationale**

**i. Summary of Information from Current Submissions**

A dose range-finding study for PND 11 rats (MRID 49067202) was performed where PND 11 juvenile rats (both sexes) were treated with gavage doses of 0.1 to 4.0 mg/kg/day for up to 11 consecutive days. One male out of 6 rats was found dead following the fourth administration at 3.2 mg/kg/day. The NOAEL was 1.6 mg/kg/day, and clinical signs (including tremor) were observed at 2.0, 2.4, and 2.8 mg/kg/day. Cholinesterase levels were not measured. The study author concluded that 2.8 mg/kg/day was a suitable high dose for repeated administration to PND 11 rats and that 3.6 mg/kg was suitable for a single dose (time to peak effect study).

A dose range-finding study for adult female rats (MRID 49067205) was performed where groups of female adult rats were treated at gavage doses of 1.75 to 7.5 mg/kg/day for up to 11 consecutive days. All animals died or were killed at 7.5 mg/kg/day. The NOAEL was 3.375 mg/kg/day, and clinical signs (including tremor) were observed at 3.75 and 5.6 mg/kg/day. Signs were rare at 3.75 mg/kg/day and common at 5.6 mg/kg/day. For example, all 5.6 mg/kg/day animals showed tremor on all days except Day 5 when 2/3 animals had tremor; conversely, the 3.75 mg/kg/day animals showed tremor on Days 6 and 7 (3/3), and 1/3 showed tremor on Day 11. Cholinesterase levels were not measured. The study author concluded that 3.75 mg/kg/day was a suitable high dose for repeated administration to young adult female rats and that 5.6 mg/kg was suitable for a single dose (time to peak effect study).

In a previous rat teratology study (Miles, Inc., Report No. MTD0108), the registrant reports that treatment with 0.85 mg/kg/day fenamiphos did not result in significant inhibition of RBC CHE in the dams and that deaths were observed in the 3 mg/kg/day dose group. This study was cited in the protocol, but was not included with the current submissions.

**ii. Summary of Applicable Information from Previous Submissions**

Due to the inadequate data concerning CHE inhibition, previously submitted studies (acute, subchronic, and developmental neurotoxicity studies) were considered to inform dose-selection by the EPA.

In an acute neurotoxicity study (MRID 44041501), single gavage doses were 0, 0.4, 1.6, and 2.4 mg/kg in each sex. RBC CHE inhibition was 24% in males and 4% in females at 0.4 mg/kg, 70% in males and 51% in females at 1.6 mg/kg, and 76% in males and 80% in females at 2.4 mg/kg. Brain cholinesterase was not affected. No deaths were observed.

The results are presented in Table 1.

**Table 1. Percent CHE Inhibition from Control in an Acute Neurotoxicity Study in Wistar Rats (50 Minutes After Dosing).** [a]

| | Males | | Females | |
|---|---|---|---|---|
| Dose (mg/kg) | RBC CHE | Brain CHE | RBC CHE | Brain CHE |
| 0.4 | -24* | +6* | -4 | +3 |
| 1.8 | -70** | +0 | -51* | +2 |
| 2.4 | -76** | -2 | -80** | -1 |

a Data extracted from information presented on page 30 in MRID 44041501.
* Statistically different from the control at p≤0.05.
** Statistically different from the control at p≤0.01.

In a subchronic neurotoxicity study (MRID 44051401), dietary doses were 0, 0.06, 0.61, and 3.13 mg/kg/day in males and 0, 0.08, 0.80, and 3.98 mg/kg/day in females. RBC CHE inhibition was evaluated in each sex at Weeks 4 and 15, while brain CHE inhibition was evaluated at Week 15. During both Weeks 4 and 15, there was an inhibition in RBC CHE inhibition of 5-25% at 0.61/0.80 mg/kg/day; the inhibition increased to 86-96% at 3.13/3.98 mg/kg/day. Brain cholinesterase was only significantly decreased in the 3.98 mg/kg/day females at Week 15 (↓12%; p≤0.01). Deaths of 4 males (and 3 replacement males) and 1 female resulted at 3.13/3.98 mg/kg/day in males/females. The results are presented in Table 2.

**Table 2. Percent CHE Inhibition from Control in a Subchronic Neurotoxicity Study in Wistar Rats.** [a]

| Dose | Males | | Females | |
|---|---|---|---|---|
| (mg/kg/day) | RBC CHE | Brain CHE | RBC CHE | Brain CHE |
| Week 4 | | | | |
| 0.06/0.08 (M/F) | +12 | - | +5 | - |
| 0.61/0.80 (M/F) | -5 | - | -25 | - |
| 3.13/3.98 (M/F) | -88** | - | -86** | - |
| Week 15 | | | | |
| 0.06/0.08 (M/F) | -6 | -5 | +13 | +5* |
| 0.61/0.80 (M/F) | -25* | -10 | -20 | -2 |
| 3.13/3.98 (M/F) | -93 [b] | -9 [b] | -96** | -12** |

a Data extracted from information presented on page 33 in MRID 44051401.
b Sample size too small for statistical evaluation.
* Statistically different from the control at p≤0.05.
** Statistically different from the control at p≤0.01.

In a developmental neurotoxicity study (MRID 46203401), fenamiphos was administered to pregnant rats in the diet at nominal dose levels of 0, 2.5, 10, or 50 ppm (equivalent to 0/0, 0.2/0.5, 0.9/2.1, and 4.8/10.3 mg/kg/day [gestation/lactation]) from gestation day (GD) 0 through lactation day (LD) 21. In the dams, RBC CHE inhibition was 15, 61, and 85% at 0.5, 2.1, and 10.3 mg/kg/day, respectively. Brain CHE inhibition was 4% at 2.1 mg/kg/day and 34% at 10.3 mg/kg/day. The pups were nursing and were only exposed to fenamiphos through the mother's milk and incidental ingestion of the dietary formulation. In the PND 4 pups, RBC CHE inhibition was 1, 4, and 20% at 0.5,

2.1, and 10.3 mg/kg/day, respectively. Brain CHE inhibition was 7% at 2.1 mg/kg/day and 9% at 10.3 mg/kg/day. In the PND 21 males, RBC CHE inhibition was 12, 16, and 61% at 0.5, 2.1, and 10.3 mg/kg/day, respectively. Brain CHE inhibition was 12% at 10.3 mg/kg/day. In the PND 21 females, RBC CHE inhibition was 1, 6, and 45% at 0.5, 2.1, and 10.3 mg/kg/day, respectively. Brain CHE inhibition was 10% at 10.3 mg/kg/day. The results are presented in Tables 3 and 4. The maternal LOAEL is 50 ppm (10.3 mg/kg/day) based on decreased body weights, body weight gains, and food consumption and increased incidence of tremors. The maternal NOAEL is 10 ppm (2.1 mg/kg/day). The offspring LOAEL is 50 ppm (10.3 mg/kg/day), based on decreased body weight and body weight gain, decrease in motor activity in males on PND 13 and inhibition of red blood cell and brain cholinesterase activity. The NOAEL is 10 ppm (2.1 mg/kg/day).

**Table 3. Percent CHE Inhibition from Control in a Developmental Neurotoxicity Study in Wistar Hannover Dams.** [a]

| Dose (mg/kg/day) [b] | Females | |
|---|---|---|
| | RBC CHE | Brain CHE |
| 0.5 | -15 | -2 |
| 2.1 | -61* | -4 |
| 10.3 | -85* | -34* |

a Data extracted from information presented on page 868 in MRID 46203401.
b Doses administered to dams in diet at lactation. Doses at gestation were 0.2, 0.9, and 4.8 mg/kg/day.
* Statistically different from the control at $p \leq 0.05$.

**Table 4. Percent CHE Inhibition from Control in a Subchronic Neurotoxicity Study in Wistar Hannover Pups.** [a]

| Dose (mg/kg/day) [b] | Males | | Females | |
|---|---|---|---|---|
| | RBC CHE | Brain CHE | RBC CHE | Brain CHE |
| PND 4 COMBINED SEXES [c] | | | | |
| 0.5 | -1 | +0 | -1 | +0 |
| 2.1 | -4 | -7 | -4 | -7 |
| 10.3 | -20 | -9 | -20 | -9 |
| PND 21 | | | | |
| 0.5 | -12 | -1 | -1 | -1 |
| 2.1 | -16 | -1 | -6 | +2 |
| 10.3 | -61* | -12* | -45* | -10* |

a Data extracted from information presented on pages 865-866 in MRID 46203401.
b Doses administered to dams in diet at lactation. Doses at gestation were 0.2, 0.9, and 4.8 mg/kg/day.
c Values apply to combined sexes rather than separately to males and females.
* Statistically different from the control at $p \leq 0.05$.

**iii. Suggested Doses:** The doses suggested by the registrant are as follows: (i) single dose study, adults: 0, 1.4, 2.8, and 5.6; PND 11 rats: 0, 1.4, 2.8, and 3.6; (ii) repeated dose study, adults: 0, 0.9, 1.9, and 3.75, PND 11 rats: 0, 0.9, 1.9, and 2.8; (iii) gestational exposure study, dams: 0, 0.25, 0.9, 1.9, and 3.0. EPA is concerned that these doses are not optimal, because mortality or serious adverse effects may be observed at the suggested high dose and the low dose may not be low enough (considering that cholinesterase inhibition can occur prior to frank clinical signs).

The EPA recommends the following gavage doses for the single and repeated dose adult studies and for the gestational exposure: 0, 0.5, 1.0, 1.8, and 2.8 mg/kg. The EPA recommends the following gavage doses for the single and repeated dose PND 11 juvenile studies: 0, 0.35, 0.7, 1.4, and 2.8.

It is preferred that range finding studies for a CCA include measurements of cholinesterase inhibition, which the submitted range finding studies (MRIDs 49067202 and 49067205) did not provide. Cholinesterase inhibition was measured in the acute neurotoxicity study at 50 minutes after dosing (time of peak effect). Cholinesterase inhibition was measured in the subchronic study during Weeks 4 and 15, by which time a steady state concentration is expected. Cholinesterase inhibition was measured in the developmental neurotoxicity study in the dams on LD 21 and in the pups on PND 4 and 21.

- **Adult:** Four doses are suggested to be tested in the adult rats, because RBC CHE inhibition is by far the most sensitive parameter for fenamiphos in adult rats; however, brain CHE inhibition is the parameter of concern for the cumulative organophosphate risk assessment. Also, inhibition greatly increases with dose. Because of these reasons, four dose groups are necessary to assess these two parameters.

  Although it is desirable to measure brain cholinesterase, the subchronic neurotoxicity study demonstrated a minor effect on brain CHE inhibition (9-12%) at 3.13/3.98 mg/kg/day, which also resulted in rat mortality. It was also noted that mortality occurred in the dams of the teratology study at 3 mg/kg/day. The acute neurotoxicity study showed no effect on brain CHE inhibition at the highest dose tested (2.4 mg/kg/day). Consequently, the high dose is suggested to be 2.8 mg/kg/day, where there may be some effect observed on brain cholinesterase without mortality (no mortality was observed at that dose in the PND 11 pup range finder, MRID 49067202). The next highest dose level is suggested to be 1.8 mg/kg/day, where 51-70% RBC CHE inhibition was observed in the acute neurotoxicity study without excessive toxicity. The lowest dose is suggested to be 0.5 mg/kg/day, which is anticipated to result in 4-25% RBC CHE inhibition in both sexes based on results of the acute and subchronic neurotoxicity studies. A dose of 1.0 mg/kg/day could be the final dose. Comparing the results from the acute neurotoxicity study and the results of the subchronic neurotoxicity study at Weeks 4 and 15, there is inadequate evidence to suggest that repeated dosing increases the severity of the symptoms. The teratology and DNT study suggests that these doses will also be appropriate for gestational exposure.
- **Pups:** Four doses are suggested to be tested for the pups, because pups were not directly treated followed by CHE inhibition measurement in any submitted study. Consequently, there is inadequate data for confidence in dose-selection; thus, more dose groups are required.

  In the range-finding study for PND 11 rats (MRID 49067202), excessive toxicity was not observed at 2.8 mg/kg/day, and this dose is recommended to be selected again for the high dose. Without any CHE inhibition data resulting from direct treatment of the PND 11 rats, this high dose will simply be sequentially diluted 1:1 with vehicle for doses of 2.8, 1.4,

0.7, and 0.35. The lowest dose is lower than with adults, because PND 11 rats are typically more sensitive to CHE inhibition than are adults. These doses should be appropriate for both single and repeated doses.

**c. Other Protocol Concerns**

Statistical comparisons should be performed, including between the adult and the PND 11 rats. It is expected that RBC and brain CHE inhibition will be measured and evaluated statistically.

Selection of litters for treatment should not involve pooling and redistribution at LD 2. Instead, the standard recommendations for a developmental neurotoxicity study (870.6300) should be followed in regards to culling the litters and selection of offspring for testing.

Unless the registrant can provide data indicating that one sex is clearly more sensitive than the other, then both sexes of adults, PND 11 rats, and fetuses must be evaluated. In the acute neurotoxicity study (MRID 44041501), RBC CHE inhibition results suggested that males were more sensitive; however, in the subchronic neurotoxicity study (MRID 44051401), RBC CHE inhibition results suggested that females were more sensitive. Obviously, these conflicting indications do not provide clarity.

**d. Dose Formulation Analyses**

Dose formulation analysis was performed for dietary mixtures and for formulation in the proposed vehicle (2% v/v Cremophor EL) for the gavage studies (MRID 49067201). The results for the dietary mixtures indicated marginal homogeneity at 1 ppm (9.4% CV) and acceptable homogeneity at 50 ppm (3.5% CV). Stability in the dietary mixtures at $21^0$C was acceptable at 1 ppm for 1 day (not tested at 2 days) and at 50 ppm for 2 days. Stability in the dietary mixtures at $-20^0$C was acceptable at 1 ppm for 46 days and at 50 ppm for 32 days (not tested at 46 days). Formulations in Cremophor EL at 0.005, 0.05, and 5 mg/mL were homogeneous (<1.7% CV) and stable up to 24 h at $21^0$C and for 15 days at $4^0$C. Of course, it is expected that dose formulations will only be used during the established duration of stability.

**e. Overall Conclusions**

The EPA recommends the following gavage doses for the single and repeated dose adult studies and for the gestational exposure: 0, 0.5, 1.0, 1.8, and 2.8 mg/kg. The EPA recommends the following gavage doses for the single and repeated dose PND 11 juvenile studies: 0, 0.35, 0.7, 1.4, and 2.8. Due to the potency of the CHE inhibitory effect, care should be taken in the accurate measurement and delivery of the doses. Dose concentration analysis should be performed. Samples will be taken at 1 hour in adults and 4 hours in pups (time of peak effect). The same method of sample preparation described in the submitted analytical study is acceptable; the dose formulations should be administered during the period where stability was demonstrated. The other protocol concerns should be addressed: (i) statistical analysis of measured data; (ii) proper allocation of pups; and (iii) testing of both sexes. If AMVAC has questions, HED is willing to discuss them via a teleconference.